



Engineered Polymerases Enable Novel Sequencing Applications

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Kapa Biosystems

- Life science reagents company focused on DNA amplification, sequencing, and molecular Dx applications
- HTP molecular evolution technology platform for engineering DNA modifying enzymes
- Developed several novel enzymes for “enzyme intensive” NGS workflows:
 - Library amplification
 - Bisulfite sequencing
 - Library preparation
 - Library quantification
 - DNA qualification

Library amplification: KAPA HiFi DNA Polymerase

Optimal enzymes for amplifying sequencing libraries



Quail M. *et al.* Nature Methods; January 2012

“The best enzyme overall for Illumina library preparation was KAPA HiFi, which performed well using either standard amplification, or a quantitative PCR premix formulation.”

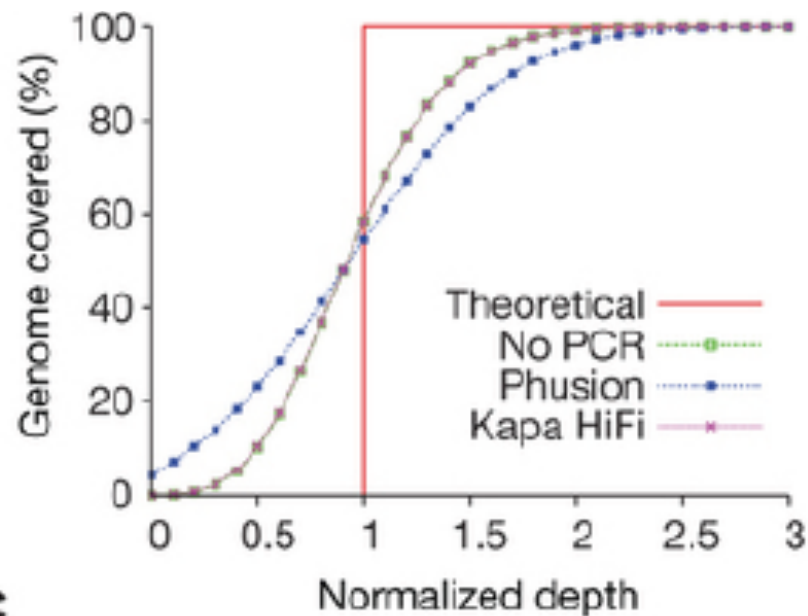
Optimizing Illumina Next-Generation Sequencing library preparation for extremely AT-biased genomes

Oyola S. *et al.* BMC Genomics; January 2012

“We show that under standard conditions, high GC content regions across chromosome 1 including telomeres were over-amplified whereas regions of high AT content were under-amplified. On the other hand KAPA HiFi amplification was close to that of a PCR free library in coverage of either extremes of base composition.”

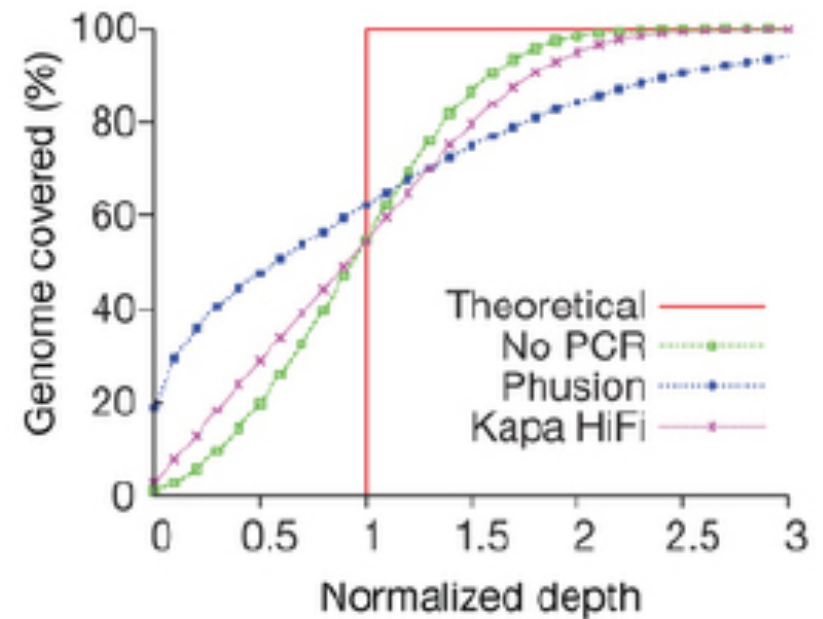
Library amplification: KAPA HiFi DNA Polymerase

B. pertrussis (68% GC)



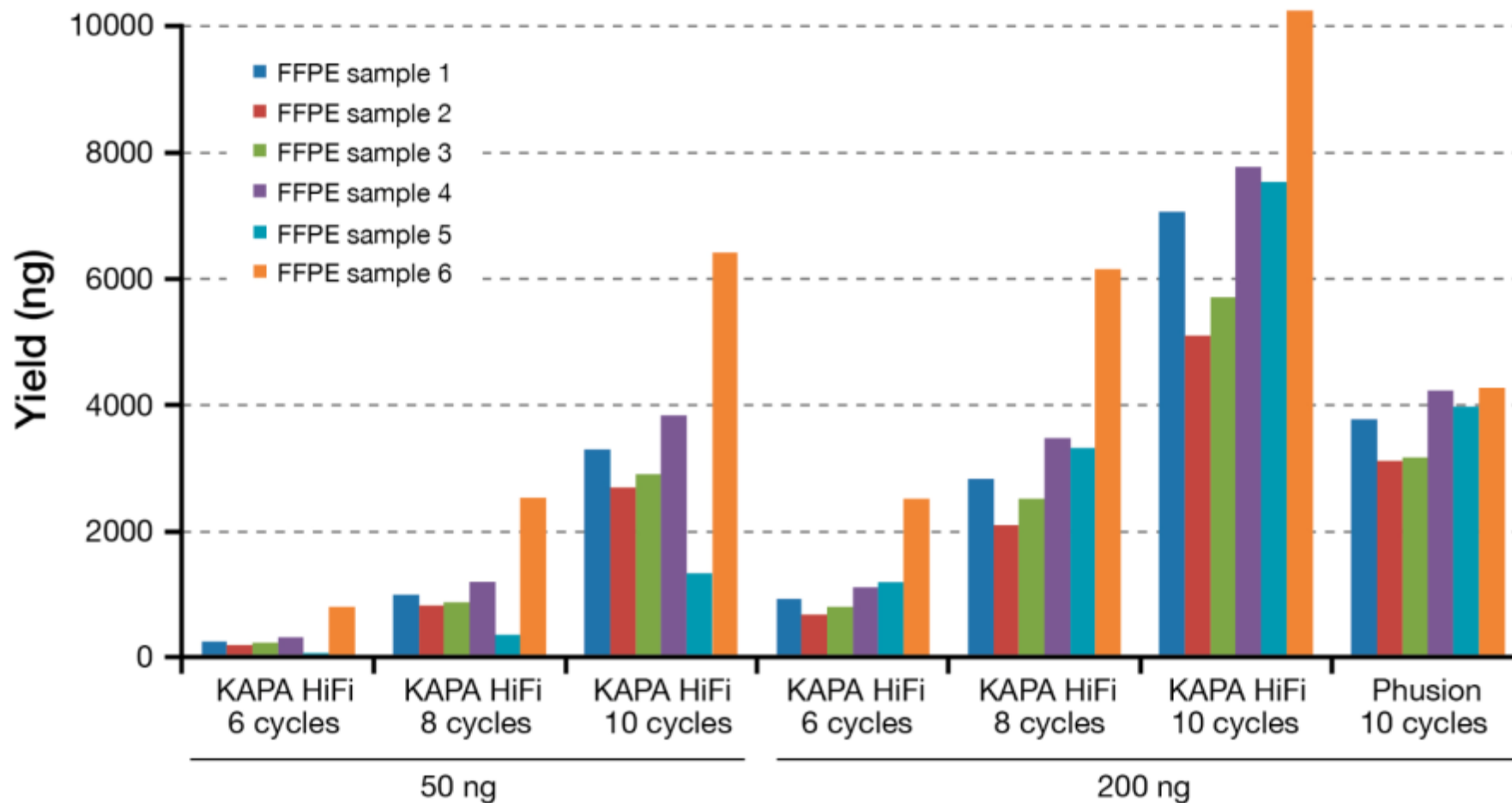
c

P. falciparum (19% GC)



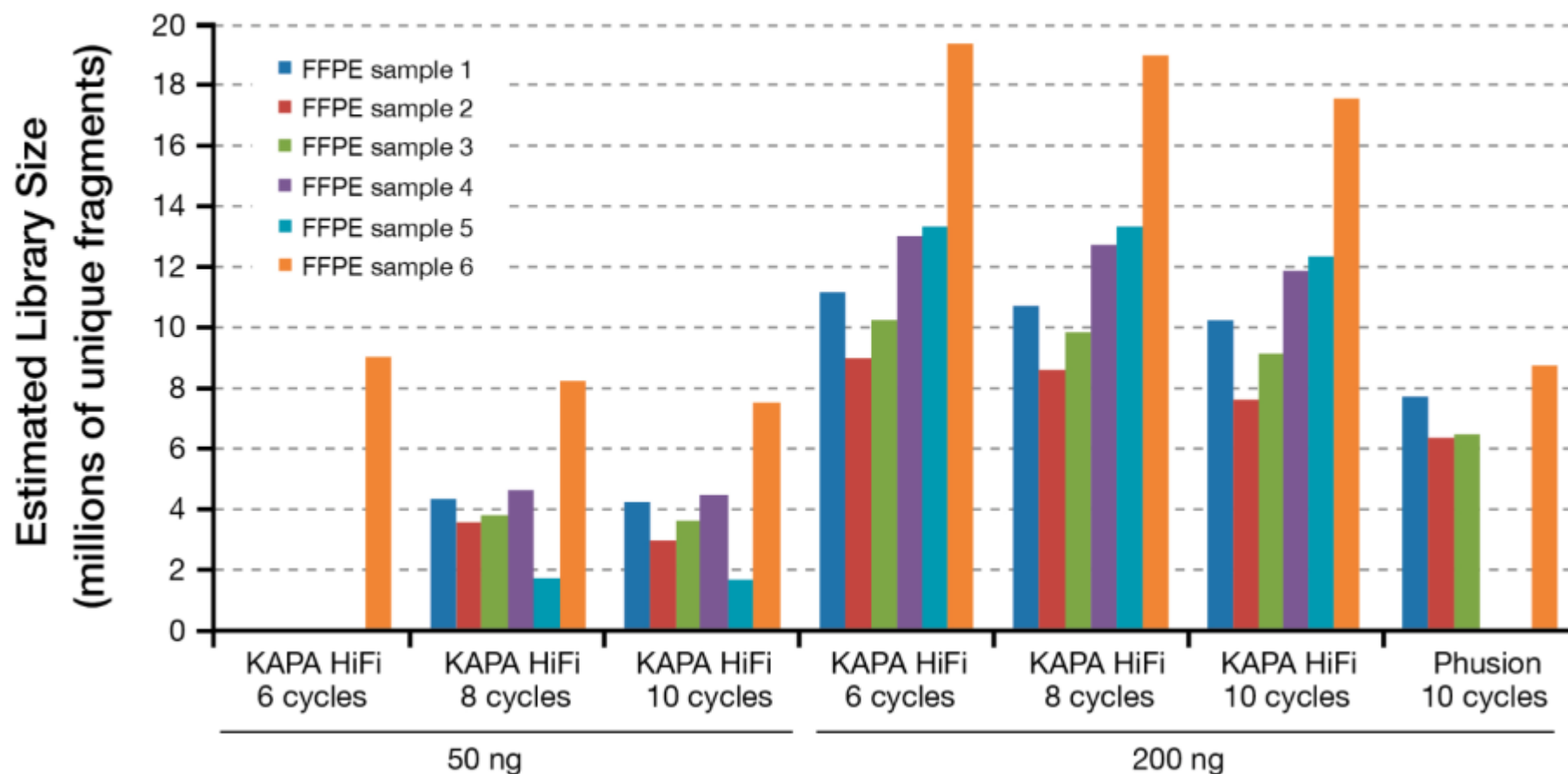
Library amplification: low-input FFPE DNA

Improved Yield



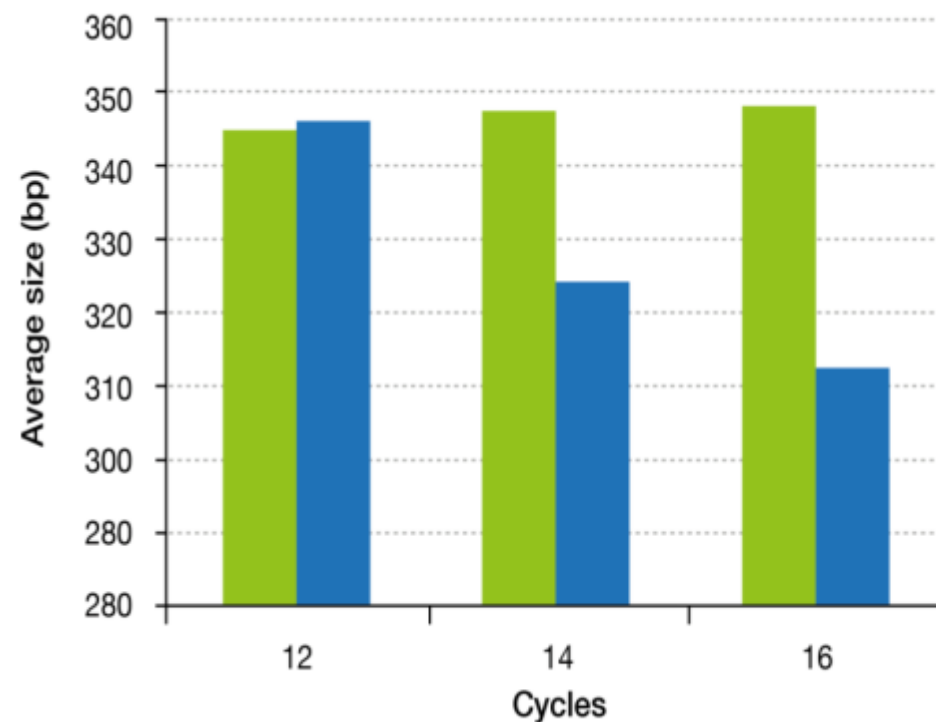
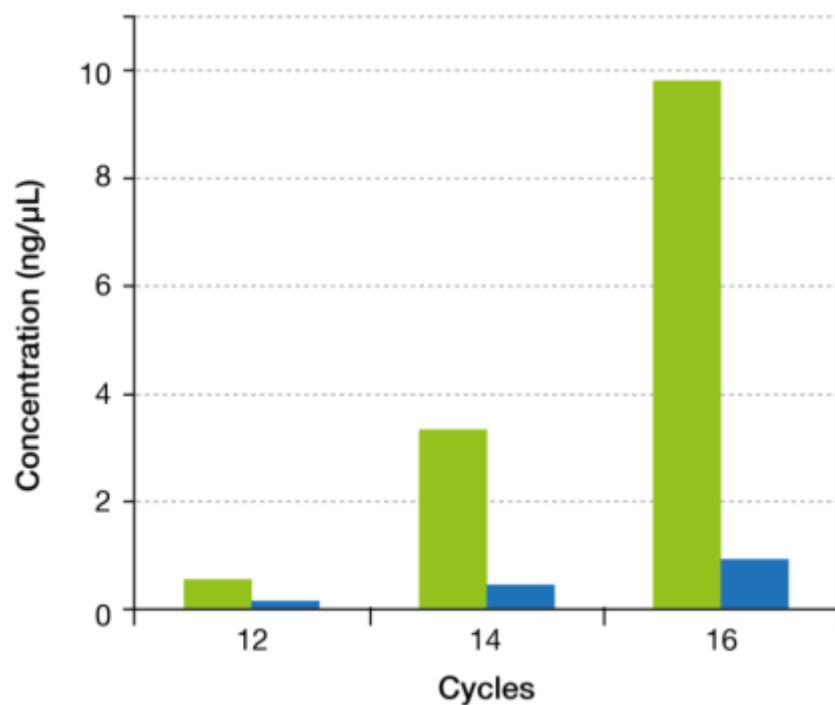
Library amplification: low-input FFPE DNA

Improved Library Diversity



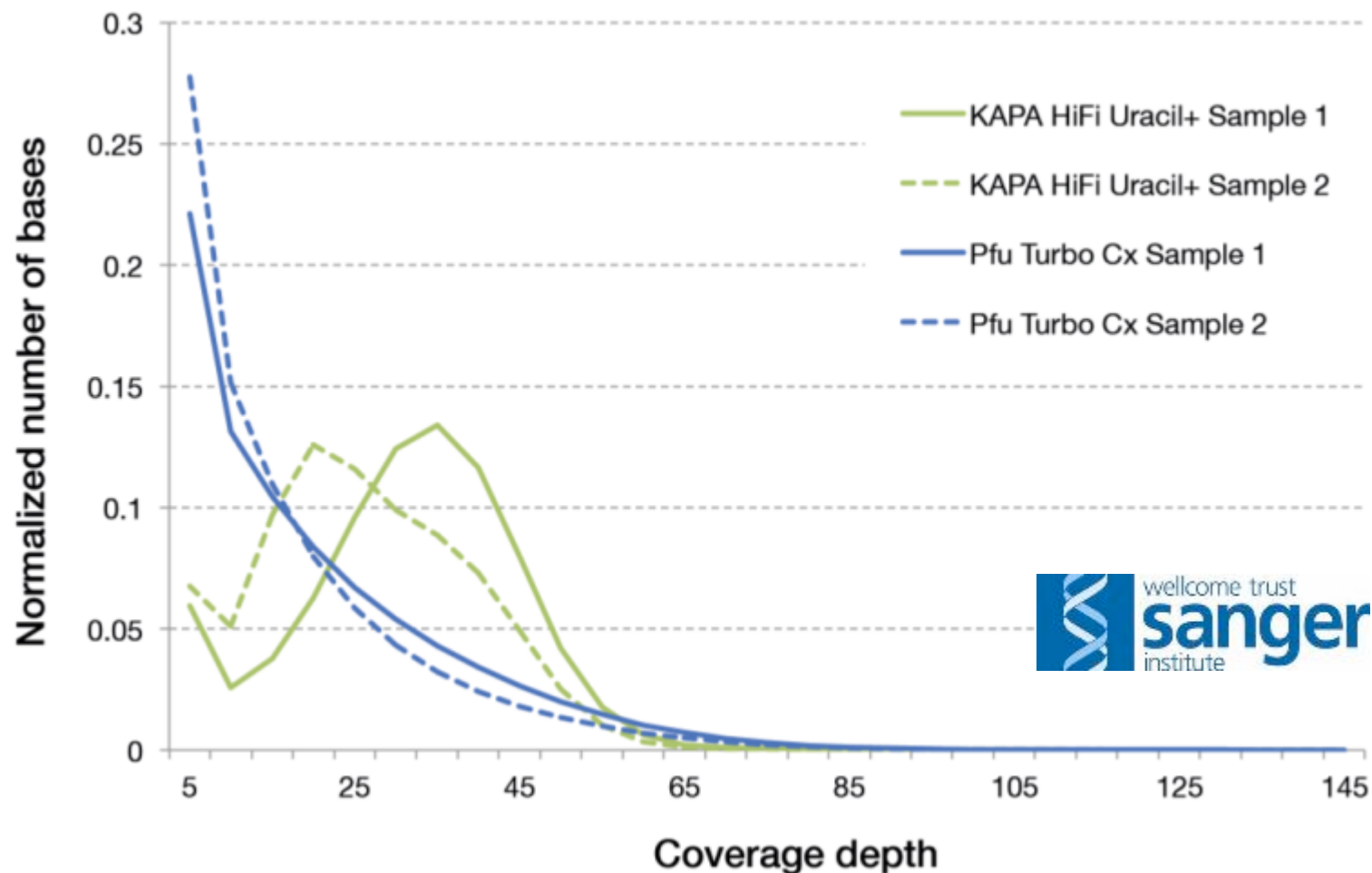
Library amplification: bisulfite-treated DNA

KAPA HiFi Uracil+ produces higher yields with minimal size bias in comparison with Agilent Pfu Turbo Cx.



Library amplification: bisulfite-treated DNA

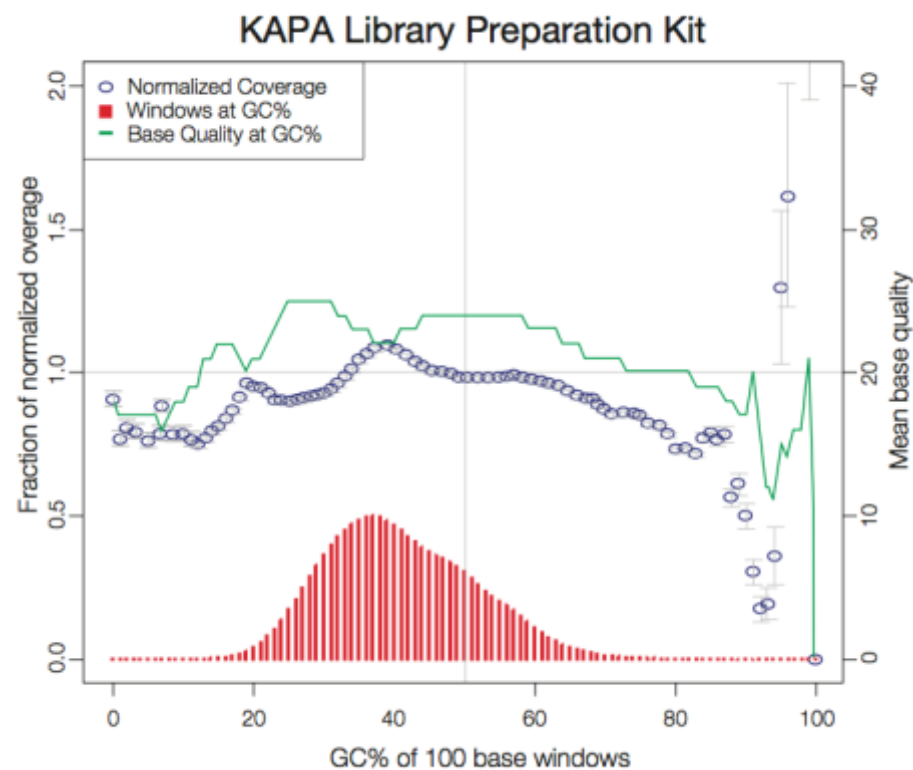
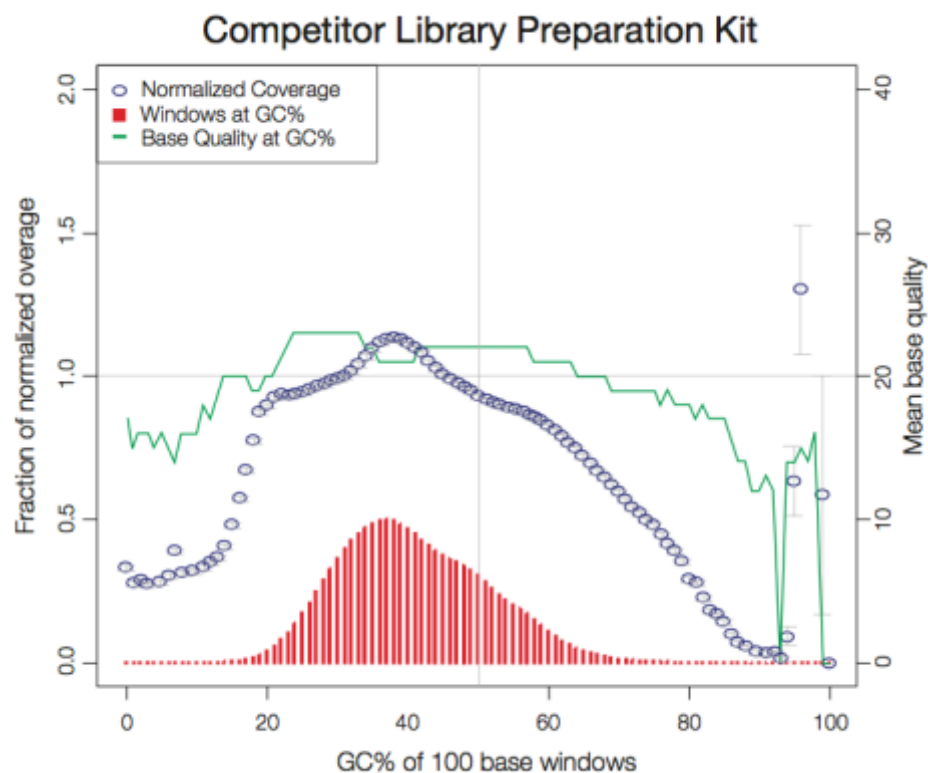
KAPA HiFi Uracil+ provides greater coverage depth uniformity.



Library preparation: reduced bias



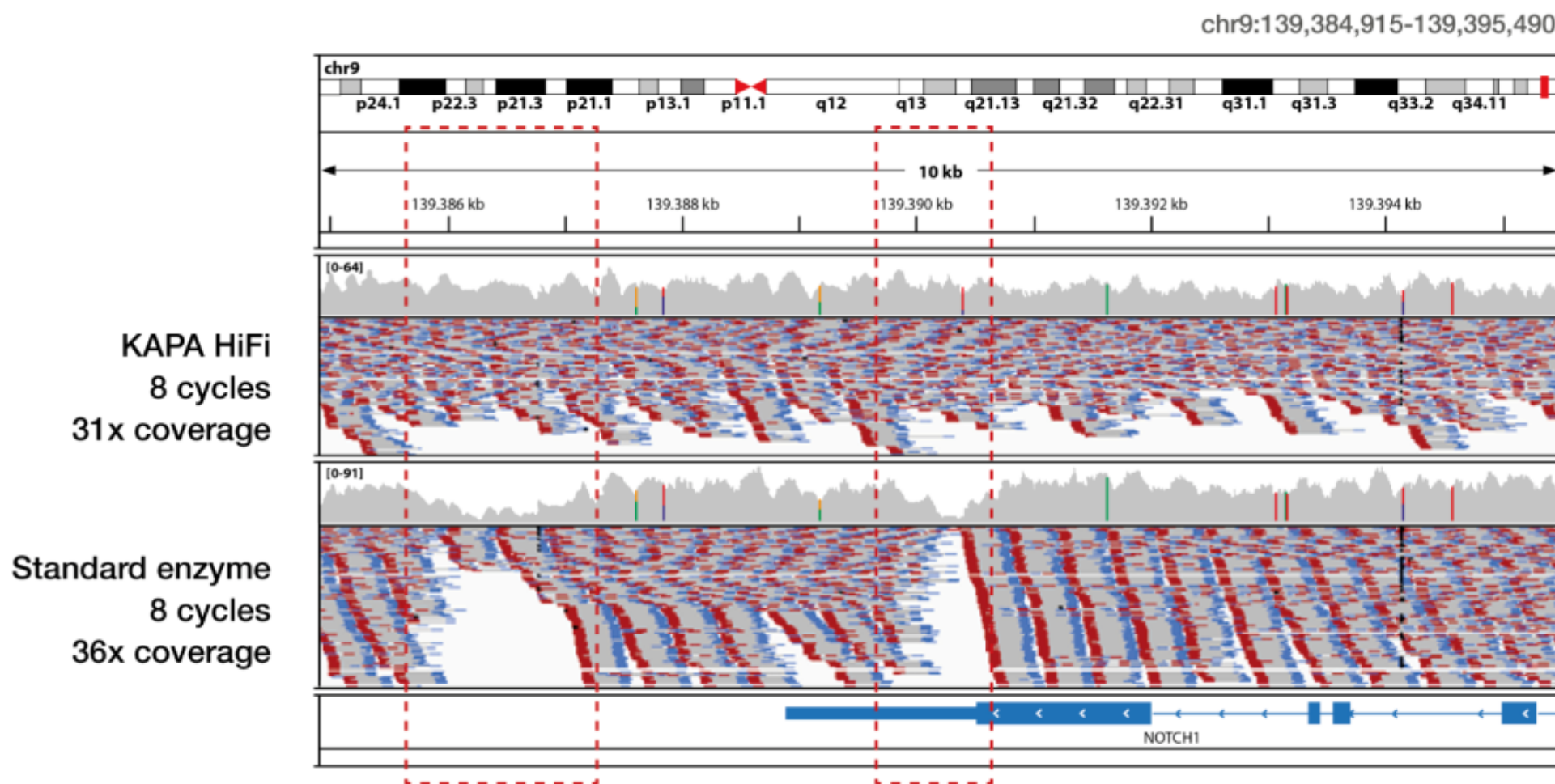
Reduced amplification bias and improved sequencing coverage in hgDNA WGS



Library preparation: high GC

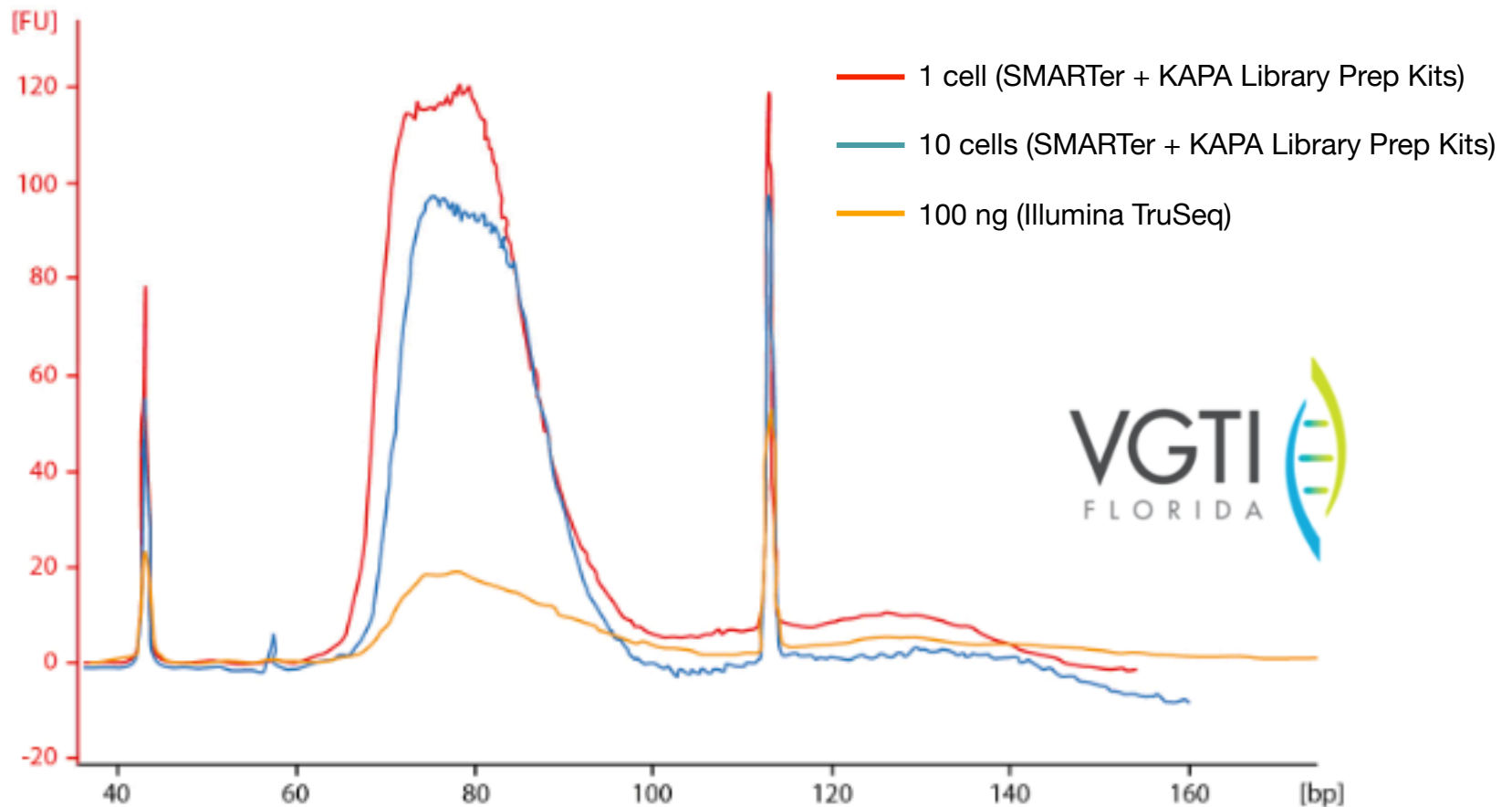


Reduced bias results in improved coverage across the NOTCH1 exon.



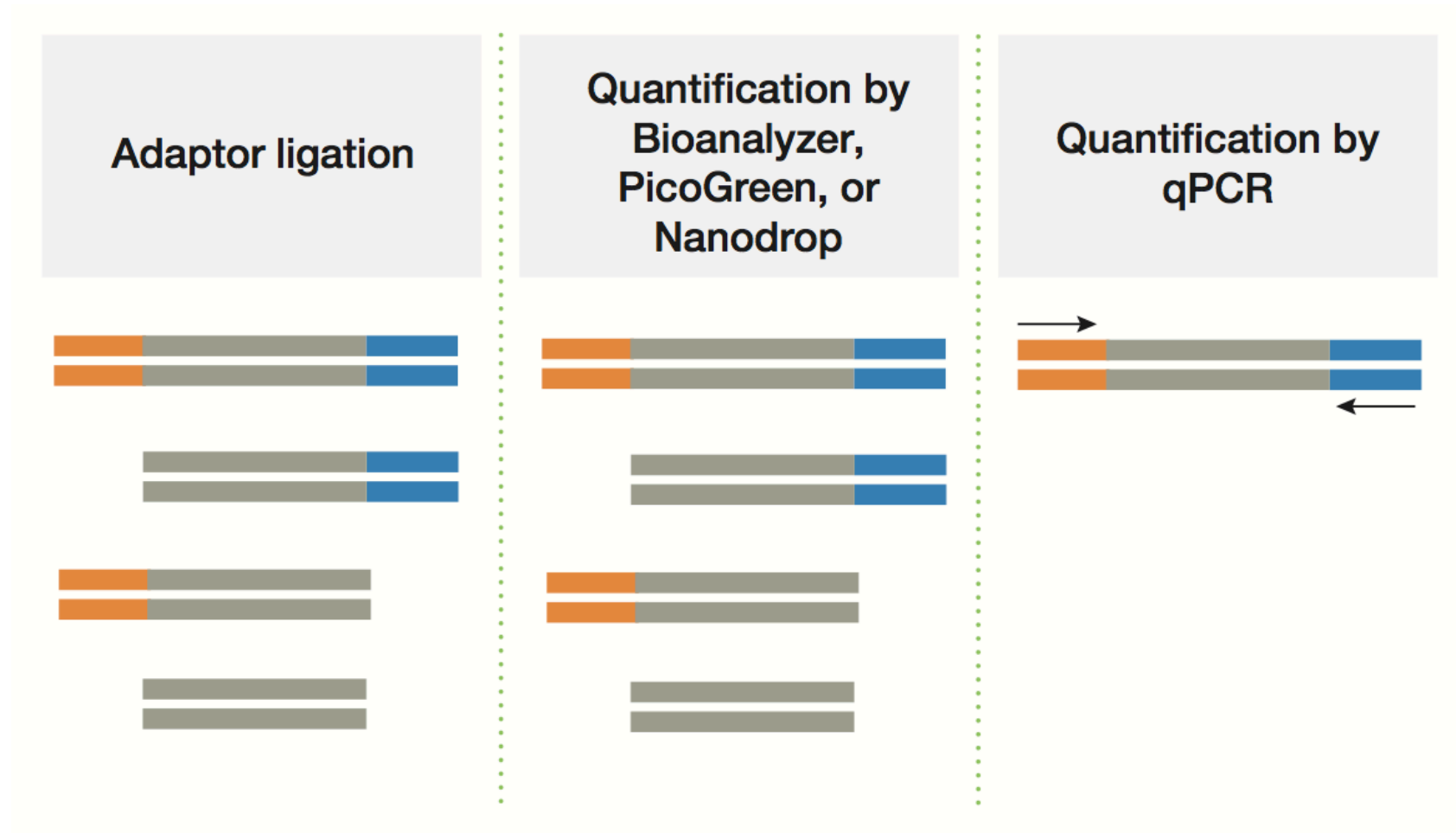
Library preparation: low input RNA-seq

In combination with Clontech SMARTer cDNA synthesis, KAPA Library Preparation Kits enable multiplexed deep sequencing from ultra-low input RNA.



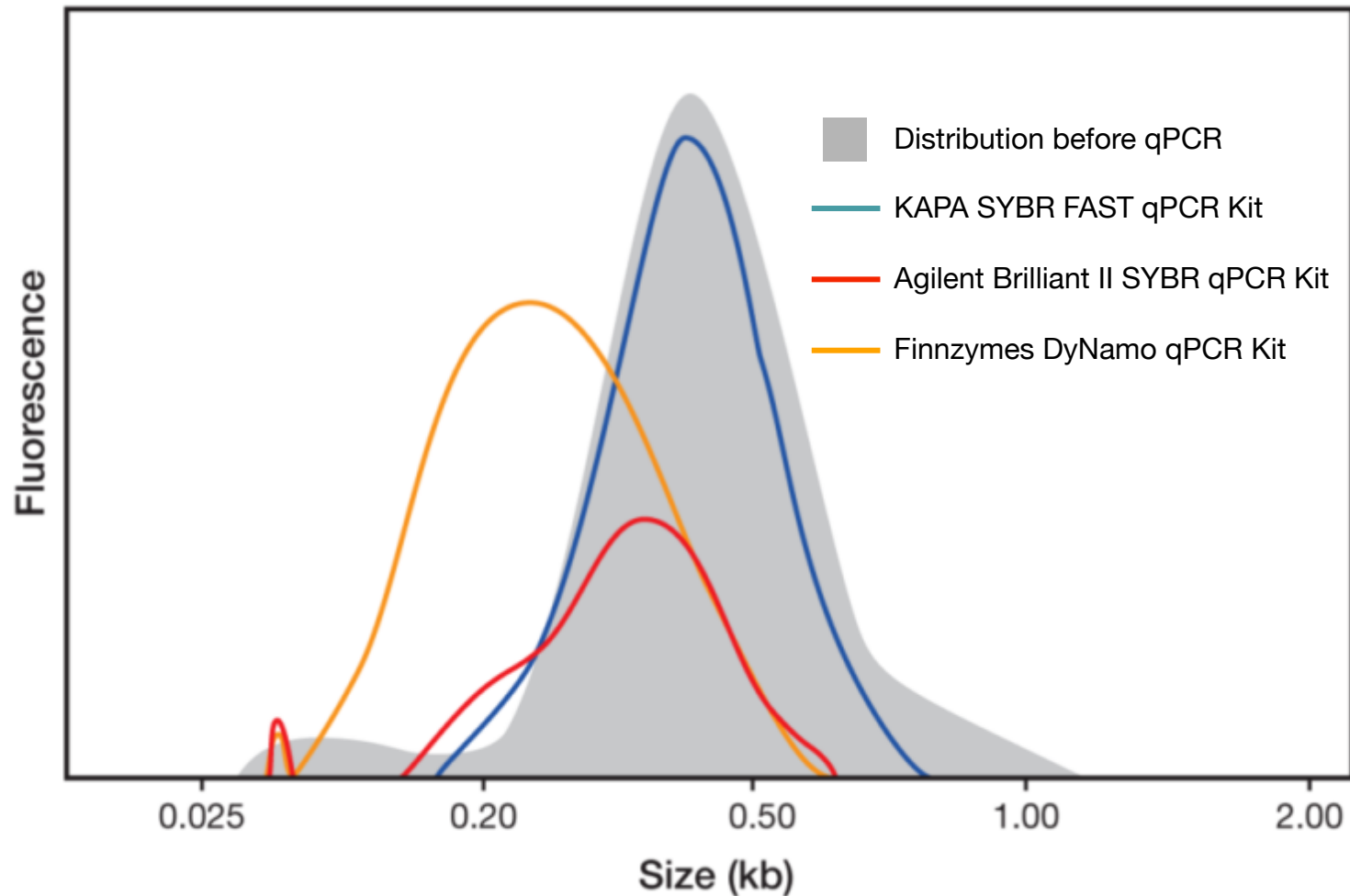
Library quantification

qPCR only quantifies PCR-amplifiable library molecules

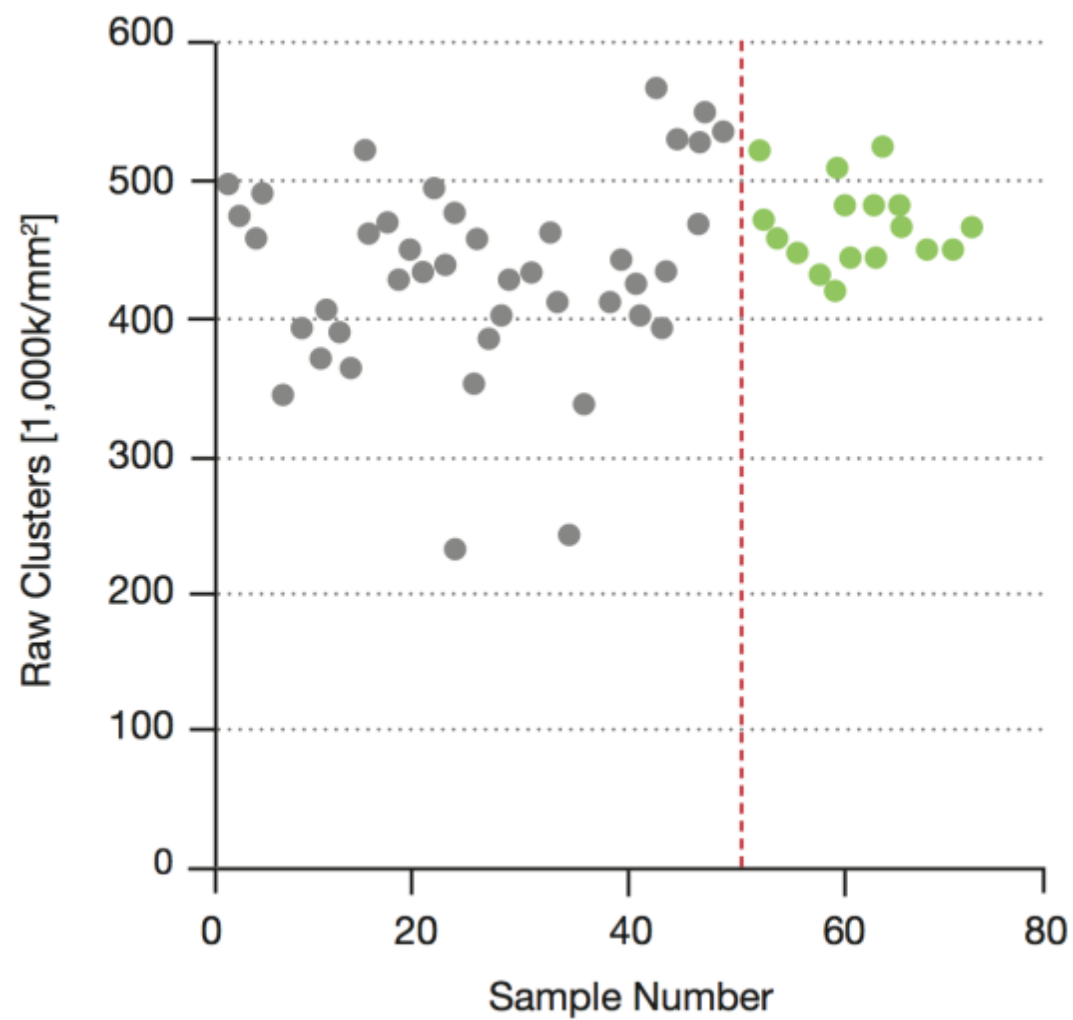


Library quantification

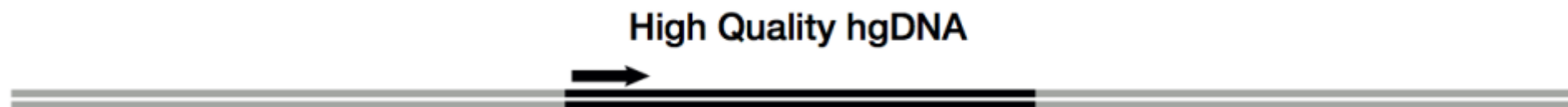
Accurate quantification without size bias of longer templates using KAPA SYBR[®] FAST qPCR Master Mix



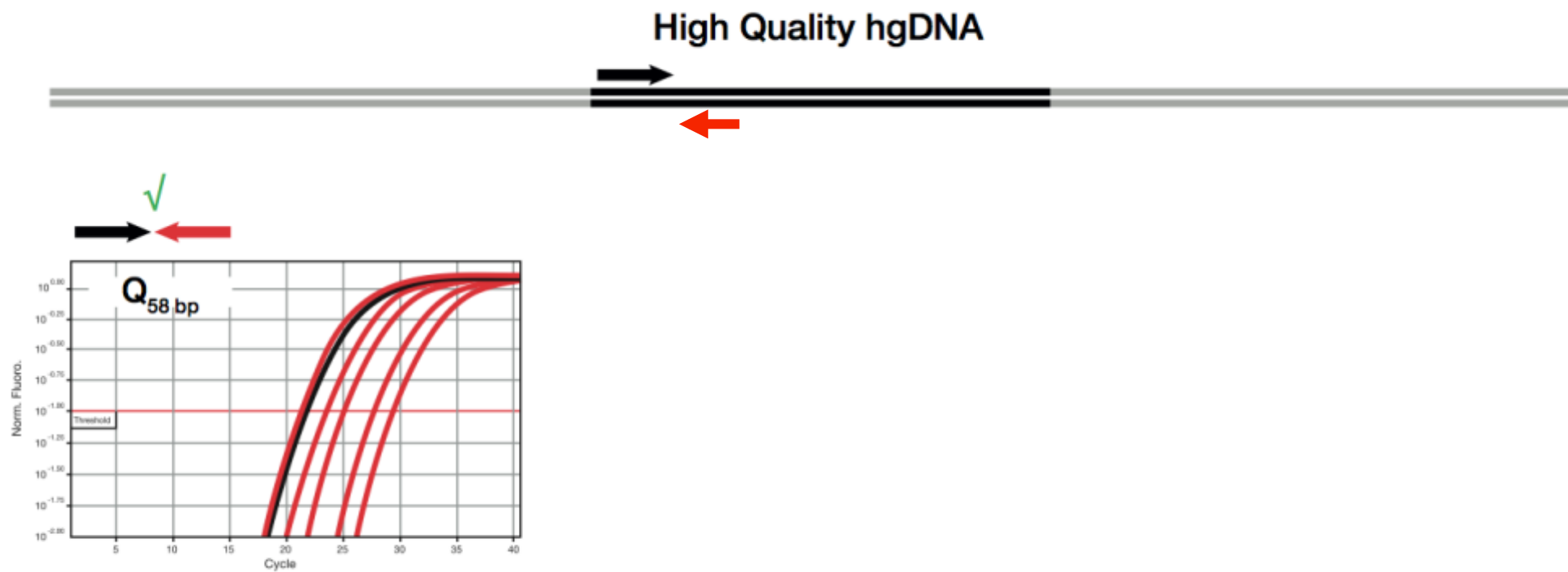
Library quantification



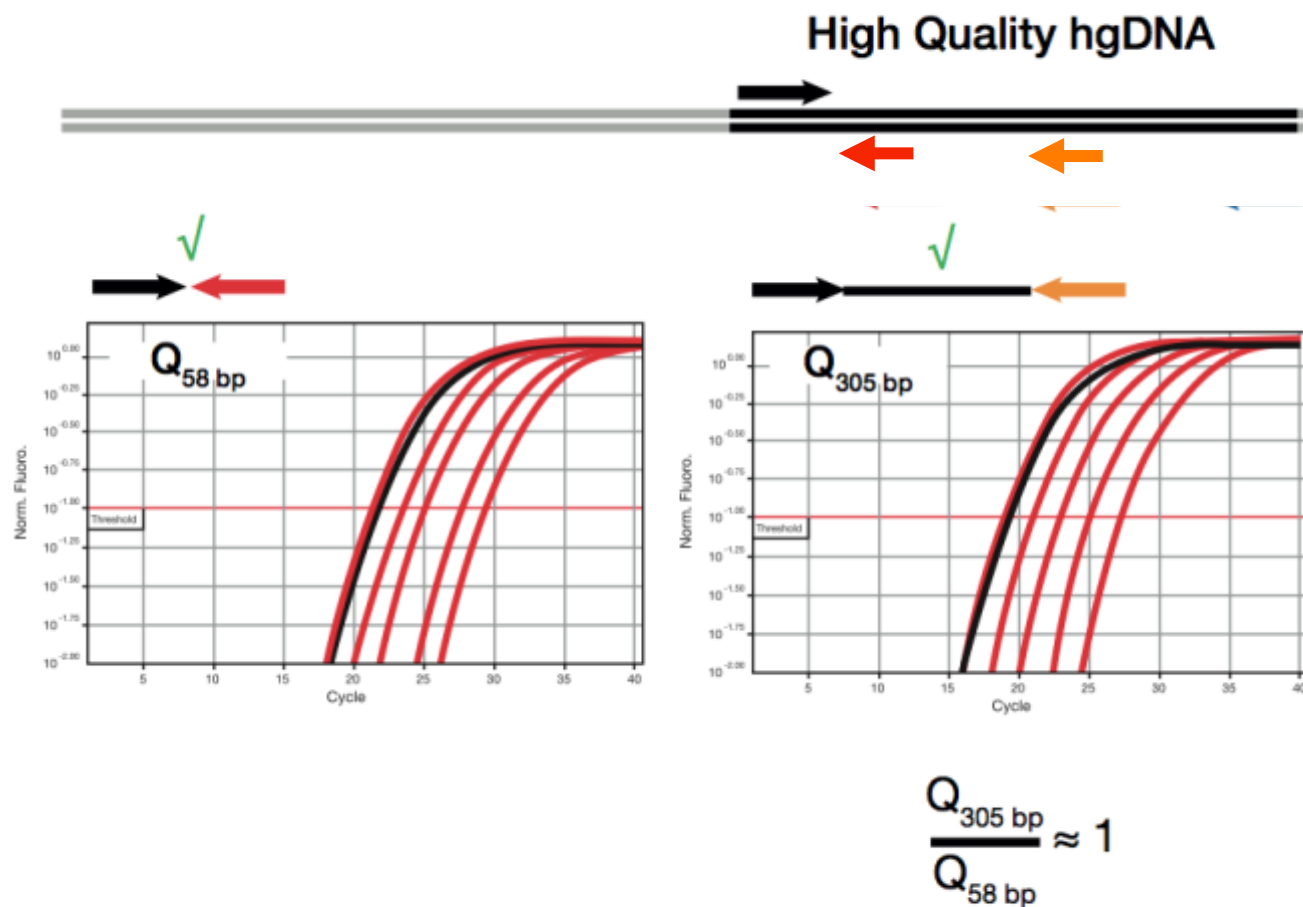
Human gDNA qualification



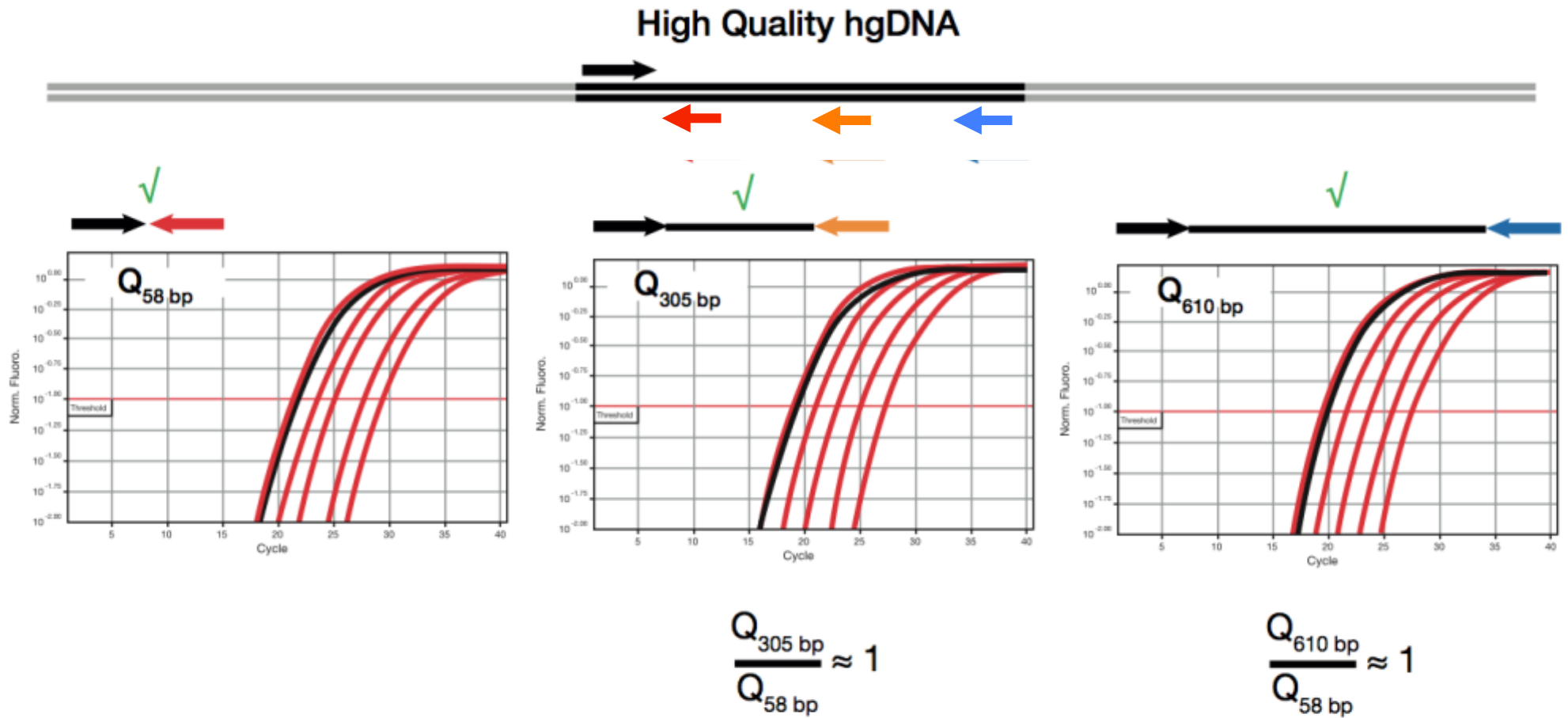
Human gDNA qualification



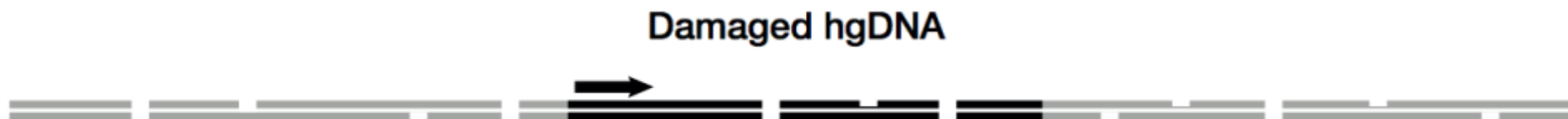
Human gDNA qualification



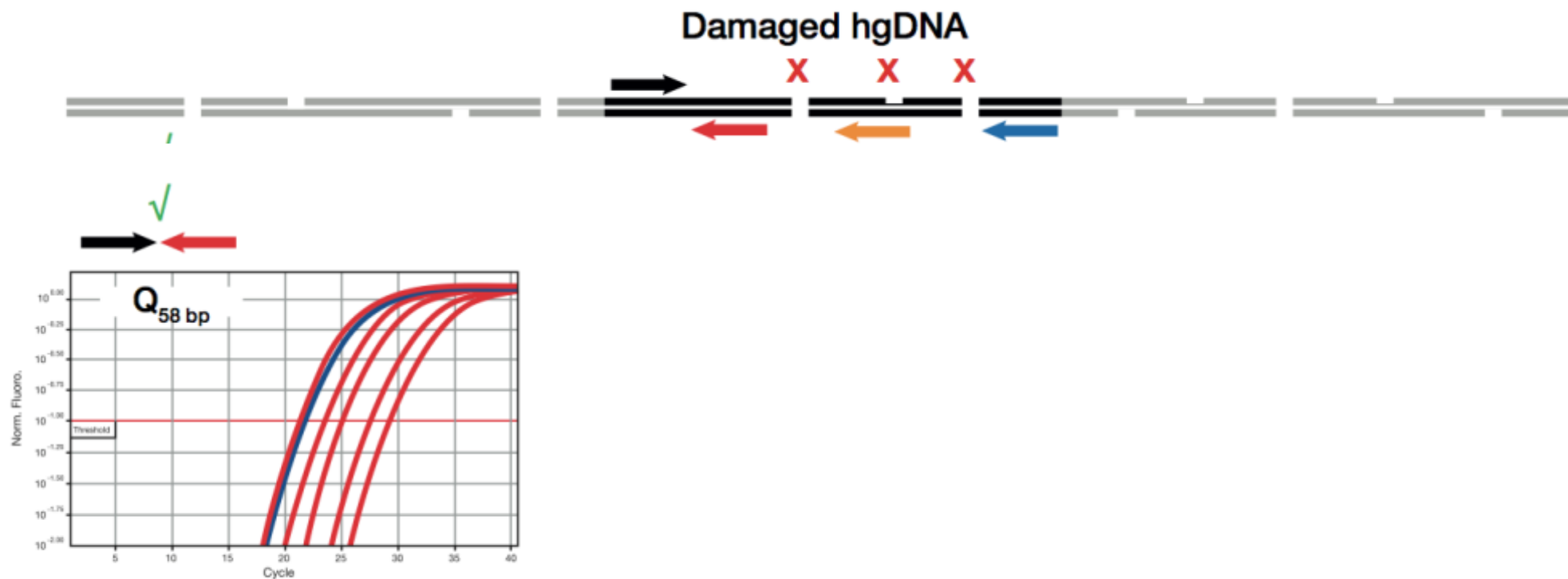
Human gDNA qualification



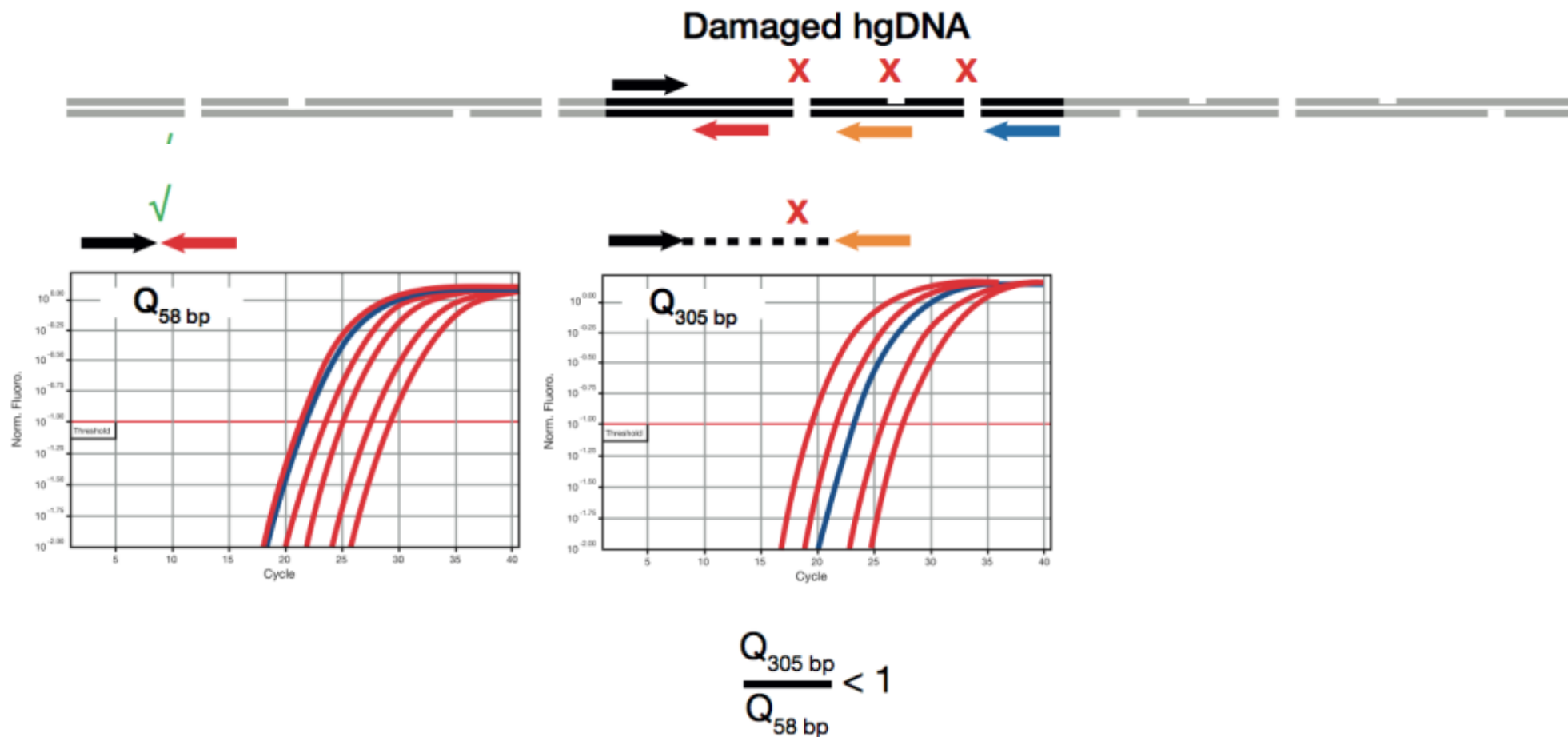
Human gDNA qualification



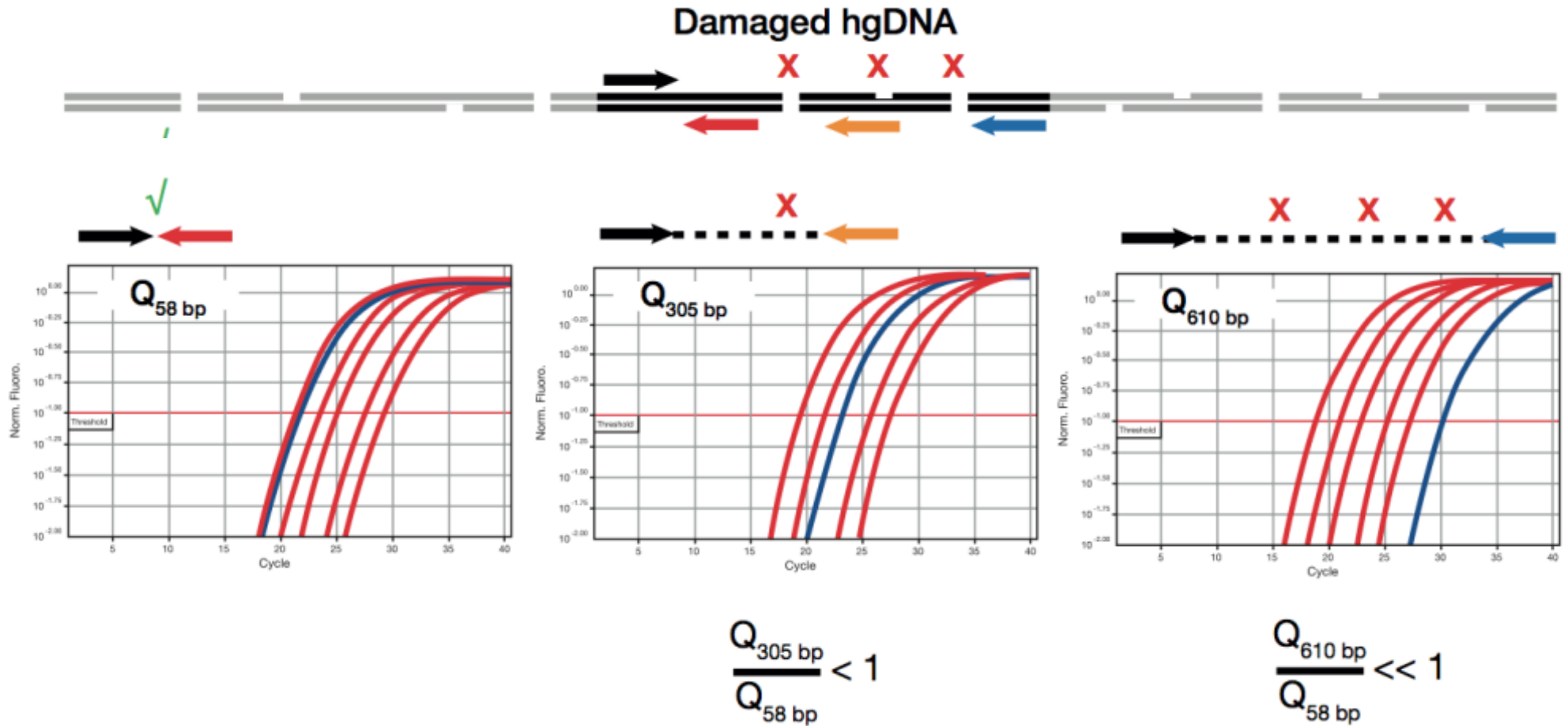
Human gDNA qualification



Human gDNA qualification

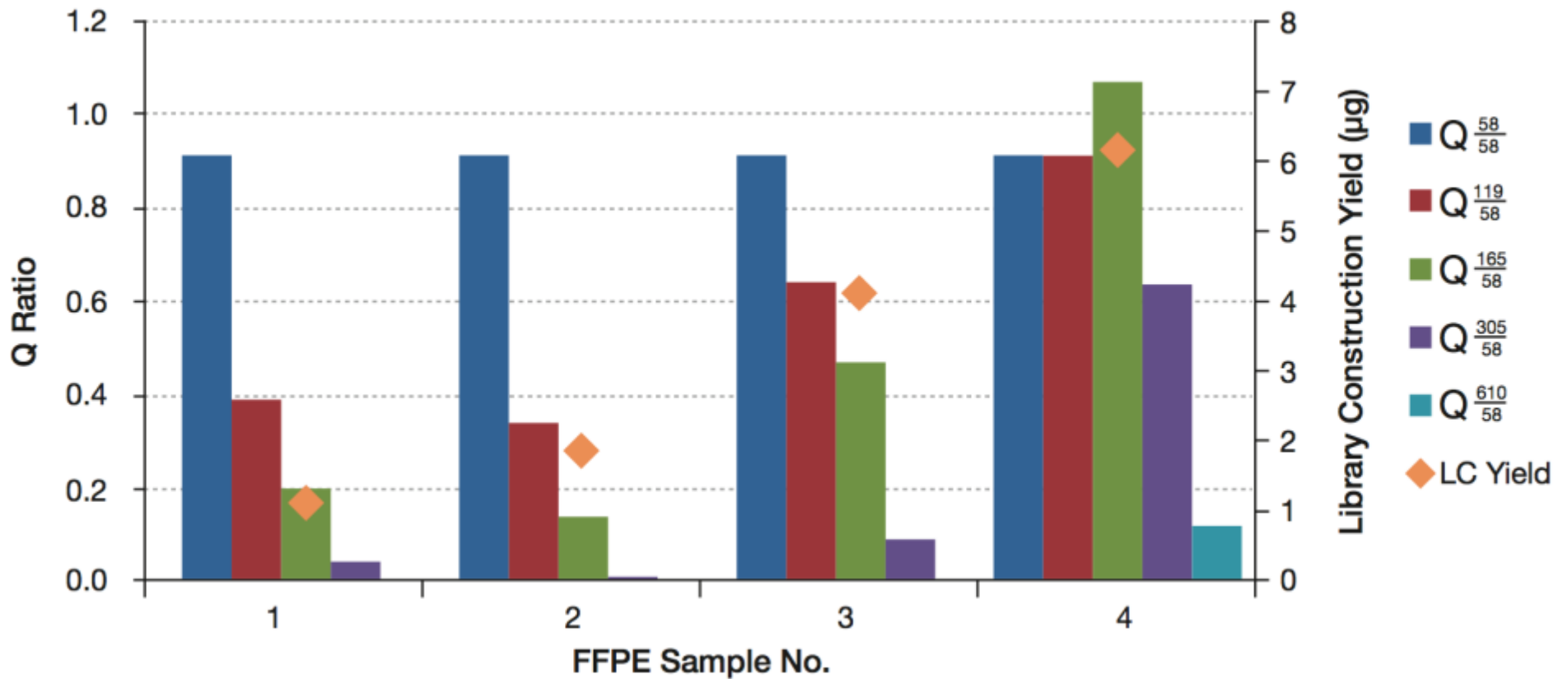


Human gDNA qualification



Human gDNA qualification

Q-ratios predict successful FFPE library construction



Complete solution for NGS sample prep

- **KAPA Library Amplification Kits**
 - Contain KAPA HiFi for high yield, low bias, and improved coverage
- **KAPA HiFi Uracil+**
 - Amplify bisulfite-treated DNA libraries with high yield, low bias, and high fidelity
- **KAPA Library Preparation Kits**
 - High efficiency and quality result in higher yields of adaptor-ligated library molecules
 - Contain KAPA HiFi Library Amplification module for improved coverage uniformity
 - Suitable for low input DNA, e.g. FFPE
 - Customized kits for automated 96 sample, “bead-in” protocols
- **KAPA Library Quantification Kits**
 - Accurately quantify only cluster generating library molecules
- **KAPA Human gDNA Qualification and Quantification Kit**
 - Assess quality of FFPE DNA
 - Predictive of library construction success



Thank You!